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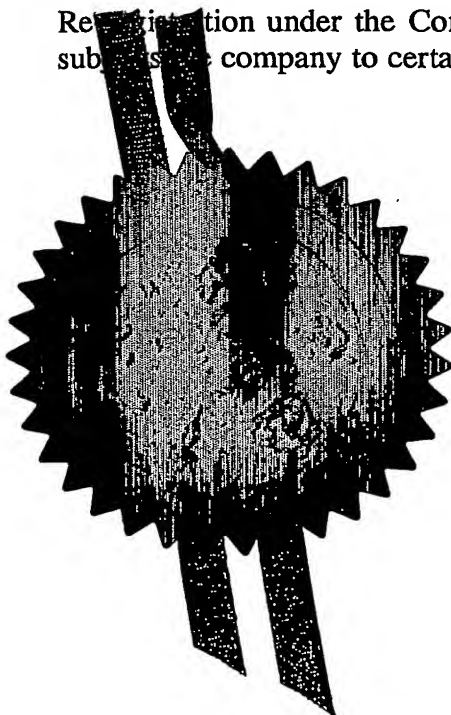
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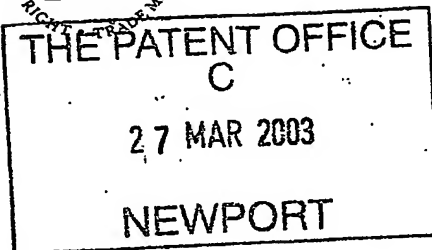
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2. Patent application number (The Patent Office will fill in this part)	27 MAR 2003	0307026.5	
3. Full name, address and postcode of the or of each applicant (underline all surnames)	Rowett Research Institute Greenburn Road Bucksburn Aberdeen AB21 9SB 7737034001		
Patents ADP number (if you know it)	04151262001		
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom		
4. Title of the invention	"Bacterial Supplement"		
5. Name of your agent (if you have one)	Murgitroyd & Company		
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	Scotland House 165-169 Scotland Street Glasgow G5 8PL		
Patents ADP number (if you know it)	1198015		
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day / month / year)
8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))	Yes		

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Continuation sheets of this form	-
Description	30
Claim(s)	-
Abstract	-
Drawing(s)	3 4 3

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Priority documents	-
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11. I/We request the grant of a patent on the basis of this application.

Murgitroyd & Co
Signature

Date
26 March 2003

Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom

Beverley Ouzman

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Figure 1.

Sequence information for five of the lactate utilising strains.

S D6 1L/1

GATGAACGCTGGCGGCGTGCCTAACACTGCAAGTCGAACGAAGCACCTTACCTGATTCTTCGGAT
GAAGGTCTGGTGACTGAGTGGCGGACGGGTGAGTAACCGTGGGTAACCTGCCCTGTACAGGGGG
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CCGCCCCGTACACCATGGGAGTCGGAAATGCCCGAAGCCAGTGACCCAACCATATGGAGGGAGC
TGTCGAAGGTGGAGCCGGTAACTGGGGTG

S 6M/1

GATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGAAGCACCTTACGAGATTCTTCGGA
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CGTCACACCATGGGAGTCGGAATGCCCAGGCCAGTGACCCAACCTTATGAAGGAAGCCnGTC
CAAGGTTGAACCCGTTAACTGGGGnnTT

Ss3/4

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Ss2/1 and Ssc/2

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ACGCCCCAAGTCAGTGACCCAACCGTAAGGAGGAGCTGCCGAAGCGGGACCGATAACTGGGGTG
AAGTCGTAACCAGGTAGCCGT

W = A or T

Y = T or C

R = G or A

N = Unknown

Bacterial Supplement

This invention relates to improvements in health and nutrition for both animals and humans following the ingestion of specific bacteria capable of utilising lactic acid.

Under normal conditions the concentration of lactic acid (lactate) in the mammalian gut is very low despite the fact that many bacterial species, such as lactobacilli, streptococci, enterococci and bifidobacteria that reside in the intestine produce this acid in large quantities as a fermentation end product. Lactic acid is also produced by host tissues.

It has been hypothesised that the accumulation of lactic acid is normally prevented by the ability of certain other bacteria that inhabit the gut to consume lactic acid and to use it as a source of energy. The identity of the microorganisms that are postulated to conduct this metabolic process in the mammalian large intestine has largely not previously

1 been elucidated, Bourriaud et al (2002). Kanauchi et
2 al (1999) revealed that a strain of *Bifidobacterium*
3 *longum* was co-incubated with a strain of *Eubacterium*
4 *limosum* on germinated barley feedstuff for three days
5 there was a marked increase in acetate formed and a
6 small increase (less than 3 mM) in butyrate formed
7 when compared to the incubations with *E. limosum*
8 alone.

9
10 In the rumen of cattle and sheep the species
11 *Selenomonas ruminantium*, *Veillonella parvula* and
12 *Megasphaera elsdenii* are regarded as the most
13 numerous utilisers of lactate (Gilmour et al., 1994;
14 Wiryawan and Brooker, 1995). The contribution of
15 *Megasphaera elsdenii* appears to be particularly
16 significant in the rumen, based on the high
17 proportion of carbon flow from lactic acid to
18 propionic acid and this species employs the acrylate
19 pathway for this purpose (Counotte et al., 1981).
20 *Megasphaera elsdenii* produces a variety of end
21 products including propionate, butyrate, caproate and
22 branched chain fatty acids from lactate (Ushida et al
23 (2002), Kung and Hession, (1995)). This probably
24 reflects the ability of this species to use lactate
25 despite the presence of other carbon sources such as
26 sugars, whereas *Selenomonas* uses lactic acid only in
27 the absence of other energy sources. This has led to
28 interest in the use of *Megasphaera* as a probiotic
29 organism that might be added to animal (Kung and
30 Hession, 1995; Ouwerkerk et al., 2002), or even human
31 diets to prevent the harmful accumulation of lactic
32 acid. In ruminant animals (cattle and sheep)

1 accumulation of lactic acid occurs when a large
2 amount of readily fermentable substrate (such as
3 starch and sugars) enters the rumen. Rapid
4 fermentation, particularly by organisms such as
5 *Streptococcus bovis*, drives down the pH, creating
6 more favourable conditions for the proliferation of
7 lactic acid producing bacteria such as lactobacilli,
8 and *S. bovis* itself. Normal populations of bacteria
9 capable of utilising lactate (lactate utilisers) are
10 unable to cope with the greatly increased production
11 of lactic acid. Unaided, lactic acid may accumulate
12 to levels that can cause acute toxicity, laminitis
13 and death (Nocek, 1997; Russell and Rychlik, 2001).
14
15 Similar events occurring in the large intestine can
16 also cause severe digestive and health problems in
17 other animals, for example in the horse where high
18 lactate levels and colic can result from feeding
19 certain diets.
20
21 In humans lactic acid accumulation is associated with
22 surgical removal of portions of the small and large
23 intestine, and with gut disorders such as ulcerative
24 colitis and short bowel syndrome (Day and Abbott,
25 1999). High concentrations of lactic acid in the
26 bloodstream can cause toxicity (Hove et al., 1994),
27 including neurological symptoms (Chan et al., 1994).
28 Much of this lactic acid is assumed to derive from
29 bacterial fermentation, particularly by
30 bifidobacteria and by lactobacilli and enterococci.
31 Lactic acid can also be produced by host tissues, but

1 the relative contributions of bacterial and host
2 sources are at present unclear.

3
4 Conversely, the formation of other acid products, in
5 particular butyric acid (butyrate), is considered to
6 be beneficial as butyric acid provides a preferred
7 energy source for the cells lining the large
8 intestine and has anti-inflammatory effects (Inan et
9 al., 2001, Pryde et al., 2002). Butyrate also helps
10 to protect against colorectal cancer and colitis
11 (Archer et al., 1998; Csordas, 1996).

12
13 We have now established a method of isolating novel
14 bacteria that are remarkably active in consuming
15 lactic acid from human faeces. Preferably the method
16 allows isolation of bacteria which convert the lactic
17 acid to butyric acid. According to this method
18 several new bacteria that are remarkably active in
19 converting lactic acid to butyric acid have been
20 isolated.

21
22 One group of these bacteria is from the newly
23 described genus *Anaerostipes caccae* (Schwiertz et
24 al., 2002). Although some main characteristics of *A.*
25 *caccae* are described in this publication, its ability
26 to use lactate was not reported and has only recently
27 been recognised as described herein.

28
29 The invention relates to a method for selecting a
30 strain of lactic acid-utilising bacteria, which
31 method comprises the steps of:

1 a) Providing (for example isolating) a
2 bacterial culture from a human faecal
3 sample;

4 b) selecting a single colony of bacteria;

5 c) growing said colony in a suitable medium
6 containing lactic acid; and

7 d) selecting a strain of bacteria consuming
8 relatively large amounts of lactic acid,
9 all of the above steps being conducted
10 under anaerobic conditions.

11

12 In the above method, the reference to "relatively
13 large amounts of lactic acid" is defined as meaning
14 the bacteria used more than 10 mM of D, L or DL
15 lactic acid during growth into stationary phase, or
16 24 hours at 37°C in YCFALG or YCFAL medium.

17

18 Preferably the strain of lactic acid utilising
19 bacteria also produces high level of butyric acid and
20 the method of the invention may therefore comprise an
21 additional step of:

22 e) selecting a strain of bacteria producing
23 relatively large quantities of butyric
24 acid.

25

26 In the above step the reference to "relatively large
27 quantities of butyric acid" is defined as meaning the
28 bacteria produces more than 10 mM of butyric acid
29 during growth into stationary phase, or 24 hours at
30 37°C in YCFALG or YCFAL medium.

31

1 Preferably the strain of lactic acid utilising
2 bacterium must be capable of converting lactate
3 produced by another gut bacterium from dietary
4 components such as resistant starch.

5
6 Preferably the lactic acid used in step c) is both D-
7 and L- isomers of lactic acid.

8
9 Preferably the suitable medium to grow bacteria is
10 nutritionally rich medium in anaerobic Hungate tubes.

11
12 Preferably the selected strain of bacteria is re-
13 purified using nutritionally rich medium in anaerobic
14 roll tubes.

15
16 A further aspect of the invention is a bacterial
17 strain that produces butyric acid as its sole or
18 predominant fermentation product from lactate and
19 which has been isolated according to the method of
20 the invention described above.

21
22 The bacteria *A. caccae* strain L1¹92 deposited at
23 NCIMB (National Collections of Industrial, Marine and
24 Food Bacteria in Aberdeen, United Kingdom) under No
25 13801^T on 4 November 2002 and at DSM under No 14662
26 on 4 November 2002.

27
28 The bacteria strain Ss2/1 *Cl. indolis*-like deposited
29 at NCIMB under No 41156 on 13 February 2003.

30

1 The bacteria strain S6M/1 of *Eubacterium hallii*
2 deposited at NCIMB under No. 41155 on 13 February
3 2003.

4
5 Another aspect of the invention is a strain of
6 bacteria having a 16S rRNA gene sequence which
7 differs at less than 3% of residues out of
8 approximately 1400 from one of the sequences shown in
9 Figure 1.

10

11 Another aspect of the invention is the use of at
12 least one of the above-mentioned bacterial strains in
13 a medicament or foodstuff.

14

15 Another aspect of the invention is a method to
16 promote butyric acid formation in the intestine of a
17 mammal which includes the administration of a
18 therapeutically effective dose of at least one of the
19 above described strains of live butyric acid
20 producing bacteria. The bacterial strain may be
21 administered by means of a foodstuff or suppository
22 or any other suitable method.

23

24 Another aspect of the invention is a method for
25 treating diseases associated with a high dosage of
26 lactic acid such as lactic-acidosis, short bowel
27 syndrome and inflammatory bowel disease, including
28 ulcerative colitis and Crohn's disease, which
29 comprises the administration of a therapeutically
30 effective dose of *Anaerostipes caccae* or at least one
31 above-mentioned strains of live lactic acid utilising

1 bacteria. Advantageously the strain selected may
2 also produce a high level of butyric acid.

3
4 Further, another aspect of the invention is a
5 prophylactic method to reduce the incidence or
6 severity of colorectal cancer or colitis in mammals
7 caused in part by high lactic acid and low butyric
8 acid concentrations, which method comprises the
9 administration of a therapeutically effective dose of
10 at least one above identified strains of live lactic
11 acid utilising bacteria and/or butyric acid producing
12 bacteria mentioned above or of *Anaerostipes caccae*.

13
14 Another aspect of the invention is the use of live
15 *Anaerostipes caccae* or at least one of the above
16 mentioned lactic acid utilising bacteria as a
17 medicament. Advantageously the strain chosen may
18 produce butyric acid as its sole or predominant
19 fermentation product from lactate. Preferably the
20 bacteria are used in the treatment of diseases
21 associated with high levels of lactic acid such as
22 lactic acidosis, short bowel syndrome and
23 inflammatory bowel disease including ulcerative
24 colitis and Crohn's disease.

25
26 According to another aspect of the invention at least
27 one lactate-utilising strain of bacteria as mentioned
28 above or *Anaerostipes caccae* are used in combination
29 with lactic acid producing bacteria including those
30 such as *Lactobacillus* spp. and *Bifidobacterium* spp.
31 or other additives or growth enhancing supplement
32 currently used as probiotics.

1 The combination of strains would potentially enhance
2 the health-promoting benefits of the lactic acid

~~3 bacterium by converting its fermentation products~~

4 (lactic acid alone or lactic acid plus acetic acid)
5 into butyrate. Indeed it is possible that certain
6 health-promoting properties currently ascribed to
7 lactic acid bacteria might actually be due to
8 stimulation of other species such as lactate-
9 consumers *in vivo*, particularly where probiotic
10 approaches (see below) are used to boost native
11 populations in the gut. Furthermore the presence of
12 the lactic acid producing bacteria in a combined
13 inoculum could help to protect the lactate consumer
14 against oxygen prior to ingestion.

15
16 The growth and activity of the novel bacteria may be
17 promoted by means of providing certain growth
18 requirements, required for optimal growth and enzyme
19 expression to the bacteria, present in the animal or
20 human gastrointestinal tract. These bacterial growth
21 enhancing nutrients are often referred to as
22 prebiotics or synbiotics.

23
24 Thus the invention provides methods to promote the
25 growth and enzyme expression of the microorganism and
26 hence removal of lactate and production of butyrate
27 *in vivo*, for example, via a prebiotic or synbiotic
28 approach (Collins and Gibson, 1999).

29
30 Another aspect of the invention is a method for
31 treating acidosis and colic in animals, particularly
32 in ruminants and horses or other farm animals, by

1 administration of a therapeutically effective dose of
2 *Anaerostipes caccae* or at least one of the lactate
3 utilising bacteria mentioned above. Advantageously
4 the bacteria can be administered as feed additives.

5
6 For the use, prevention or treatment of conditions
7 described herein, the bacteria or prebiotic(s) or
8 synbiotic(s) are preferentially delivered to the site
9 of action in the gastro-intestinal tract by oral or
10 rectal administration in any appropriate formulae or
11 carrier or excipient or diluent or stabiliser. Such
12 modes of delivery may be of any formulation included
13 but not limited to solid formulations such as tablets
14 or capsules; liquid solutions such as yoghurts or
15 drinks or suspensions. Ideally, the delivery
16 mechanism delivers the bacteria or prebiotic or
17 synbiotic without harm through the acid environment
18 of the stomach and through the rumen to the site of
19 action within the gastro-intestinal tract.

20 Another aspect of the invention is the use of at
21 least one bacterial strain mentioned above or
22 *Anaerostipes caccae* in a method to produce butyric
23 acid from lactate and acetate. The method includes
24 the fermentation of the above described microorganism
25 selected for both their lactic acid utilising and
26 butyric acid producing abilities in a medium rich in
27 lactate and acetate. The method can be used in
28 industrial processes for the production of butyrate
29 on a large scale.

30

31

1 Brief description of the Figure

2

3 Figure 1

4

5 Sequence information for five of the lactate
6 utilising strains.

7

8 DETAILED DESCRIPTION

9

10 The experimental work performed shows the following:

- 11 1. Certain human colonic anaerobic bacteria,
12 including *A. caccae* strains, are strong and
13 efficient utilisers of lactic acid.
- 14 2. Certain human colonic anaerobic bacteria,
15 including *A. caccae* strains, are strong and
16 efficient producers of butyric acid.
- 17 3. Certain human colonic anaerobic bacteria,
18 including *A. caccae* strains, convert lactic acid
19 to butyric acid.

20

21 Example 1: Isolation and characterisation of bacteria

22

23 The bacterial strains that were isolated at RRI were
24 selected as single colonies from a nutritionally rich
25 medium in anaerobic roll tubes as described by
26 Barcenilla et al. (2000). The isolates were grown in
27 M2GSC broth and the fermentation end products
28 determined. Butyrate producing bacteria were re-
29 purified using roll tubes as described above.

30 Strains L1-92, S D8/3, S D7/11, A2-165, A2-181, A2-
31 183, L2-50 and L2-7 were all isolated using this
32 medium. Omitting rumen fluid and/or replacing the

1 sugars with one additional carbon source such as DL
2 lactate increased the selectivity of the roll tube
3 medium and this medium was used to isolate strain S
4 D6 1L/1. Strains G 2M/1 and S 6M/1 were isolated
5 from medium where DL-lactate was replaced with
6 mannitol (0.5%). Separately, non-rumen fluid based
7 media routinely used for isolating *Selenomonas* sp.,
8 namely Ss and Sr medium (Atlas, 1997) was used to
9 isolate other strains. Inoculating Sr medium roll
10 tubes with dilutions of faecal samples resulted in
11 the isolation of strain Sr1/1 while the Ss medium
12 resulted in the isolation of strains Ss2/1, Ss3/4 and
13 Ssc/2.

14
15 Example 2: *A. caccae* and other human colonic
16 bacterial isolates consumes lactic acid and acetic
17 acid and produces butyric acid when grown in rumen
18 fluid

19
20 Table 1 summarises the fermentation products formed
21 by twelve strains of anaerobic bacteria when grown
22 under 100% CO₂ in a rumen fluid-containing medium
23 containing 0.5% lactate (M2L) or 0.5% lactate, 0.2%
24 starch, 0.2% cellobiose and 0.2% glucose (M2GSCL) as
25 the energy sources. Ten of these strains were
26 isolated at RRI from human faeces as described above.
27 Strains 2221 and NCIMB8052 are stock collection
28 isolates not from the human gut and are included for
29 comparison. Table 1 demonstrates that three strains,
30 L1-92 (*A. caccae*), SD6 1L/1 and SD 6M/1 (both *E.*
31 *hallii* -related) all consumed large amounts of
32 lactate (>20mM) on both media examined, M2L and

1 M2GSCL, and produced large quantities of butyric
2 acid. *A. caccae* L1-92 in particular consumed large
3 ~~amounts of lactate and produced large amounts of~~
4 butyrate. Acetate is also consumed by all three
5 strains. The other 9 butyrate producing bacteria
6 tested either consumed relatively small amounts of
7 lactate, or consumed no lactate, on this medium.

8
9 Table 1. Comparison of human faecal isolates for the
10 ability to utilise (negative values) or produce
11 (positive values) lactate on a rumen fluid based
12 medium (M2) supplemented with lactate (M2L) and
13 lactate plus glucose, cellobiose and soluble starch
14 (0.2% each) (M2GSC).

1 Table 1

Strain ID	Closest relative	Medium	Formate	Acetate	Butyrate	Lactate
S D8/3	Adhufec 406**	M2L	1.15	0.97		-3.94
"		M2GSCL	21.66	0.77	10.88	6.43
S D6 1L/1	<i>E. hallii</i>	M2L		-19.74	35.48	-32.41
"		M2GSCL		-9.78	22.58	-21.85
S 6M/1	<i>E. hallii</i>	M2L	0.79	-19.01	31.73	-23.72
"		M2GSCL	1.31	-5.06	22.77	-28.42
G 2M/1	HucA19*	M2L		2.82	7.97	23.66
"		M2GSCL		0.01	12.94	9.52
S D7 1L/1	ND#	M2L		0.51	0.08	7.58
"		M2GSCL	1.85	0.43	4.84	-10.25
2221	<i>But. Fibrisolvens</i>	M2L	1.37	-3.61	1.57	21.57
"		M2GSCL	19.4	-5.57	18.02	11.75
8052	<i>Cl. acetobutylicum</i>	M2L		-12.42	19.31	-0.87
"		M2GSCL	0.13	-1.79	18.00	-5.80
A2-165	<i>F. prausnitzii</i>	M2L	1.98	0.62	3.56	2.94
"		M2GSCL	17.47	-6.97	18.38	-5.65

Strain ID	Closest relative	Medium	Formate	Acetate	Butyrate	Lactate
A2-183	Roseburia sp.	M2L		0.86	1.84	10.63
"		M2GSCL	-0.15	-12.70	18.23	5.33
A2-181	Roseburia sp.	M2L	0.58	-0.26	1.75	
"		M2GSCL	0.33	-11.05	18.68	5.22
L2-50	Coprococcus sp.	M2L	1.06	2.32	0.52	0.43
"		M2GSCL	19.37	4.47	7.60	3.41
L1-92	Anaerostipes caccae	M2L		-29.42	37.00	-25.60
"		M2GSCL	0.63	-27.03	44.78	-45.48

1

2

3 * clone library sequence, uncultured (Hold et al., 2002)

4 + clone library sequence, uncultured (Suau et al., 1999)

5 # ND not determined

6

1 **Example 3: *A. caccae* and other human colonic**
2 bacterial isolates consumes lactic acid and acetic
3 acid and produces butyric acid when grown in rumen
4 fluid free medium

5
6 Table 2 (a) shows the utilisation and production of
7 formate, acetate, butyrate, succinate and lactate, on
8 this occasion performed using the rumen fluid-free
9 medium YCFA (Duncan et al. 2002) containing no added
10 energy source, or with 32 mM lactate (YCFAL) or
11 lactate plus 23 mM glucose (YCFALG) as added energy
12 sources. Separately table 2 (b) reveals the levels
13 of the two isomers of lactate (D and L) remaining at
14 the end of the incubations and the concentration of
15 glucose metabolised during the incubations. Five
16 additional new lactate-utilising isolates were
17 discovered using the semi-selective medium as
18 described earlier and are included in Tables 2 (a)
19 and (b), although one of these (Ss 3/4) proved to
20 consume a relatively small amount of lactate only on
21 the YCFAL medium (Table 2a). Analysis of the
22 consumption of the D and L isomers reveals that three
23 strains (Ss2/1, Ssc/2 and Srl/1) preferentially
24 consumed D lactate. Partial repression of lactate
25 consumption by glucose was observed on this medium
26 with *A. caccae* L1-92, and almost complete repression
27 for SD D6 1L/1 and Ss 3/4. The previously isolated
28 *E. hallii* strain L2-7 (Barcenilla et al., 2000)
29 behaved in a similar manner to SD D6 1L/1. The
30 higher glucose concentration in this medium compared
31 with M2GSCL is likely to explain the difference in
32 behaviour of *A. caccae* compared with Table 1. The

1 remaining five strains showed no evidence of
2 repression of lactate utilisation in the presence of
3 glucose although it is possible they use the glucose
4 before switching to lactate. Butyrate levels
5 exceeding 30mM were obtained for four strains on
6 YCFALG medium.

7
8 Table 2a. Fermentation products formed or utilised (U
9 as indicated by minus values) by human gut isolates
10 incubated on yeast extract-casitone-fatty acids
11 medium (YCFA); YCFA supplemented with lactate
12 (YCFAL); and YCFA supplemented with glucose and
13 lactate (YCFALG). The initial concentration of
14 glucose added to the medium was 23 mM and 32 mM
15 lactate was added that contained 15.5 mM L-lactate.
16 ^a Strain identity is based on 16S rRNA sequence
17 information (% identical residues with closest
18 relative is shown). See appendix 1 for sequence
19 information.

20
21 All strains except 2221 and 8052 (Table 1) isolated
22 at RRI.

Table 2a

Strain ID	Closest relative ^a	Isolation Medium	Medium	Formate	Acetate P/U	Butyrate	Succin	Lactate P/U
Ss2/1	<i>Cl. indolis</i> (95%)	Selenomonas selective	YCFA	0.02±0.04	-4.25±4.68	2.24±0.26		0.39±0.03
			YCFAL	0.18±0.02	-12.51±1.27	12.98±0.19		-15.27±2.53
			YCFALG	10.10±1.05	-24.32±1.03	35.69±1.13		-13.95±2.70
Sr 1/1	HucB 12*	Selenomonas ruminantium	YCFA		-5.42±1.77	2.33±0.03		0.36±0.12
			YCFAL	0.76±0.19	-13.35±2.27	14.15±0.17		-15.04±0.89
			YCFALG	9.53±2.03	-22.47±1.40	35.77±1.50		-13.71±0.40
S D6 1L/1	<i>E. hallii</i> HucA 15*	M2 + 0.5% lactate	YCFA		-4.96±3.26	1.42±0.23		
			YCFAL		-18.51±0.96	21.06±1.06		-29.93±0.60
			YCFALG		-9.22±2.52	20.78±1.52		-2.43±0.70
S 6M/1	<i>E. hallii</i> (98%)	M2 + 0.5% mannitol	YCFA	0.09±0.03	-2.61±2.36	1.42±0.05		
			YCFAL	0.21±0.1	-7.20±2.08	6.54±0.43		-6.27±1.27
			YCFALG	20.68±	-10.95±	29.2±		-25.82±

Strain ID	Closest relative ^a	Isolation M�dium	Medium	Formate	Acetate P/U	Butyrate	Succin	Lactate P/U
SS 3/4	HuCA19* (New species to be named)	Selenomonas selective	YCFA		4.75�2.20	6.10�0.27		1.09�0.47
			YCFAL		6.68�2.09	6.19�0.34		-9.78�2.56
			YCFALG	0.54�0.13	5.06�4.28	8.66�0.53		3.86�1.09
SSc/2	Cl. indolis (95%)	Selenomonas selective	YCFA	0.25�0.04	-0.16�1.32	2.37�0.09		0.48�0.03
			YCFAL	0.36	-12.12	13.49		-13.78
			YCFALG	10.98�1.27	-25.35�2.87	36.10�0.49		-13.34�1.28
L1-92	A. cacc�e (type strain)	M2GSC	YCFA	0.00�0.08	-2.35�2.03	1.99�0.09		
			YCFAL	-0.05�0.10	-21.98�2.45	23.35�1.16		-28.92�0.54
			YCFALG	1.49�0.13	-26.83�0.58	36.81�3.61		-12.01�1.32
L2-7	E. hallii	M2GSC	YCFA	0.02�0.01	-1.58�1.73	0.63�0.03		0.00�0.00
			YCFAL	1.09�1.55	-14.77�0.93	22.58�0.76		-30.47�0.00
			YCFALG	3.93�3.38	12.78�0.94	5.80�0.97		1.67�0.47

1

2 * clone library sequences, uncultured (Hold et al., 2002)

Table 2b. Total lactate (mM) remaining in the tubes at the end of the 24 h incubation period and separately the concentration of the two forms D and L. Total glucose (gluc) metabolised during growth also recorded (mM).

Strain number	Closest relative	Medium	Total lact.	L-lact	D-lact	Gluc used
Ss2/1	<i>Cl. indolis</i> (95%)	YCEFA	0.84±0.02			
		YCFAL	17.08±2.53	16.07±0.40	1.01±2.15	
		YCFALG	18.40±2.70	15.90±1.06	2.50±3.30	22.1±0.0
Sr 1/1	Huc B12*	YCEFA	0.81±0.12			
		YCFAL	17.31±0.89	15.05±0.34	2.26±0.68	
		YCFALG	18.64±0.40	16.37±0.79	2.27±0.71	22.0±0.2
S D6 1L/1	<i>E. hallii</i> Huc A15*	YCEFA	0.00±0.00			
		YCFAL	2.42±0.60	0.21±0.10	2.21±0.51	
		YCFALG	29.92±0.07	10.65±0.69	19.27±0.79	22.1±0.1
S 6M/1	<i>E. hallii</i> (98%)	YCEFA	0.00±0.00			
		YCFAL	26.08±1.27	9.94±0.50	16.14±1.06	

Strain number	Closest relative	Medium	Total lact.	L-lact	D-lact	Gluc used
		YCFALG	6.57±0.16	4.02±2.26	2.55±2.32	22.1±0.1
Ss 3/4	HucA19* (new species to be named)	YCFA	1.54±0.47			
		YCFAL	22.58±2.55	16.56±0.12	6.02±2.65	
		YCFALG	36.21±1.09	16.95±0.87	19.26±1.91	16.6±0.6
Ssc/2	A. caccae (L1-92)	YCFA	0.96±0.08			
		YCFAL	22.39±6.63	15.40±0.78	6.99±6.10	
		YCFALG	19.01±1.28	15.08±0.93	3.93±0.68	22.2±0.0
L1-92	A. caccae (type strain)	YCFA	0.0±0.0			
		YCFAL	3.43±0.54	1.84±0.85	1.59±0.87	
		YCFALG	20.34±1.32	8.63±0.72	11.71±2.01	
L2-7	E. hallii	YCFA	0.00±0.00			
		YCFAL	0.00±0.00			
		YCFALG	31.93±0.47	15.43±0.12	16.50±0.30	11.99±0.71

1

2 * clone library sequence, uncultured (Hold et al., 2002)

1 Table 3a. Fermentation profiles for *Bifidobacterium*
 2 *adolescentis* L2-32 and three lactate utilisers when
 3 incubated alone or in co-culture for 24 hours at
 4 37°C on modified YCFA medium (modified to contain
 5 0.1% casitone) containing 0.2% soluble starch.

6

Culture/ co-culture	Formate	Acetate	Butyrate	Total Lactate	L-Lactate
L2-32	4.29±0.92	51.04±5.44	0	5.00±0.09	5.16±0.45
L1-92	0.01±0.01	34.99±0.93	1.57±0.26	0.40±0.69	0
SD6M/1	0	35.25±2.15	0.75±0.06	0.27±0.27	0
L2-7	0.04±0.06	35.70±0.44	0.83±0.02	0	0
L2-32+L1-92	4.29±0.04	44.82±1.13	7.62±0.66	0.61±0.53	0
L2- 32+SD6M/1	4.81±1.08	48.17±6.47	6.23±1.15	0	0
L2-32+L2-7	5.16±1.37	43.88±3.74	7.35±0.27	0.36±0.01	0

7

1 Table 3b. Total viable counts (cfu per ml) of
 2 *Bifidobacterium adolescentis* L2-32 and three
 3 lactate utilisers following 24 hours at 37°C in
 4 monoculture and co-culture. *Bifidobacterium*
 5 *adolescentis* L2-32 was selected for on MRS + 0.25%
 6 propionate roll tubes and the butyrate
 7 producing/lactate utilisers were selected for on M2
 8 + 0.5% lactate roll tubes following incubation for
 9 24 hours at 37°C.

Culture / Co-culture	<i>B. adolescentis</i> L2-32	Butyrate producer / lactate utiliser
L2-32	3.8×10^8	
L1-92		2.4×10^8
SD6M/1		1.0×10^7
L2-7		8.0×10^6
L2-32+L1-92	6.4×10^8	1.7×10^9
L2-32+SD6M/1	3.8×10^8	6.8×10^8
L2-32+L2-7	3.2×10^8	5.4×10^9

Summary

11
 12
 13
 14 *A. caccae* strain L1-92 was able to consume up to
 15 30mM DL lactate, along with 20-30 mM acetate during
 16 batch culture incubation for 24 hours at 37°C with
 17 the production of >20mM, and up to 45mM butyrate;
 18 this occurred also when glucose was added as an
 19 alternative energy source (Table 1). Lactate or
 20 lactate plus glucose thus resulted in very much
 21 higher production of butyrate than observed with
 22 23mM glucose alone, when only <15mM butyrate was
 23 formed. Furthermore none of the 74 strains

1 screened previously by Barcenilla et al. (2000)
2 produced more than 25mM butyrate when tested in
3 M2GSC medium. Lactate consumption is not a general
4 characteristic of butyrate-producers, and six of
5 the strains screened in Table 1 failed to consume
6 lactate in M2GSCL medium.

7
8 Six further strains that are highly active lactate
9 utilisers (defined for example as net consumption
10 of at least 10mM of lactate during growth to
11 stationary phase or for 24 hours in YCFALG or YCFAL
12 medium at 37°C - see Table 2) were obtained
13 following deliberate screening of new human faecal
14 isolates for lactate utilisation. At least two of
15 these (SD6 1L/1 and S6M/1 - Tables 1, 2) are
16 related to *Eubacterium hallii*. (Table 2a), based on
17 determination of their 16S rDNA sequences. These
18 isolates again consume large quantities of lactate
19 and produce high levels of butyrate *in vitro*. With
20 one exception where considerable glucose repression
21 occurred (strain SD6 1L/1), significant lactate
22 utilization occurred in the presence of glucose
23 (Table 2). Three strains (Ss 2/1, Sr 1/1 and
24 Ssc/2) showed preferential utilization of D-
25 lactate, whereas the two *E. hallii*-related strains
26 SD 6M/1, SD6 1L/1 and *A. caccae* L1-92 utilise both
27 isomers (Table 2b). The two stereoisomers differ
28 in their toxicity in the human body; with the D-
29 isomer being regarded as the more toxic (Chan et
30 al., 1994, Hove et al., 1995). The present
31 invention thus provides a means of utilising both D

1 and L lactate isomers or preferentially utilising
2 D-lactate in preference to L-lactate.

3
4 *A. caccae* and newly isolated bacteria related to
5 *E. hallii* and *Cl. indolis* were shown to consume up
6 to 30mM DL, D or L lactate, along with 20-30 mM
7 acetate during batch culture incubation and convert
8 this energy in to production of at least 20mM, and
9 up to 45mM butyrate. Furthermore, these strains
10 were shown to convert all of the L-lactate produced
11 by a starch-degrading strain of *Bifidobacterium*
12 *adolescentis* into butyrate when grown in culture.
13 This is the first documentation demonstrating the
14 conversion of lactate to butyrate by human colonic
15 bacteria, some of which are likely to be new
16 species.
17

References

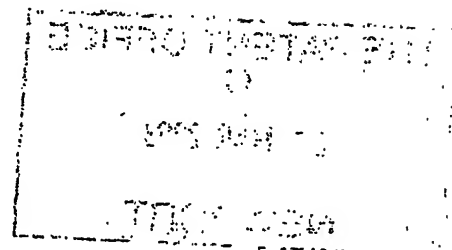
1. Archer, S.Y., Meng, S.F., Sheh, A. and Hodin, R.A. (1998). p21 (WAF1) is required for butyrate mediated growth inhibition of human colon cancer cells. Proc. Natl. Acad. Sci. USA, 95, 6791-6796.
2. Atlas, R.M. (1997). Handbook of microbiological media (2nd edition). Ed. L.C. Park. CRC Press, Cleveland, Ohio.
3. Barcenilla, A., Pryde, S.E., Martin, J.C., Duncan, S.H., Stewart, C.S., Henderson, C. and Flint, H.J. (2000). Phylogenetic relationships of dominant butyrate producing bacteria from the human gut. Appl. Environ. Microbiol., 66, 1654-1661.
4. Bourriaud, C., Akoka, S., Gouptry, S., Robins, R., Cherbut, C. and Michel, C. (2002). Butyrate production from lactate by human colonic microflora. Reprod. Nutr. Develop., 42, (Suppl. 1). S55.
5. Chan, L., Slater, J., Hasbargen, J., Herndon, D.N., Veech, R.L. and Wolf, S. (1994). Neurocardiac toxicity of racemic D, L-lactate fluids. Integr. Physiol. Behav. Sci., 29, 383-394.

- 1 6. Collins, M.D. and Gibson, G.R. (1999).
2 Probiotics, prebiotics, and synbiotics:
3 approaches for modulating the microbial
4 ecology of the gut. Am. J. Clin. Nutr., 69
5 (suppl), 1052S-1057S.
6
7 7. Counotte, G.H.M., Prins, R.A., Janssen,
8 R.H.A.M., DeBie, M.J.A. (1981). Role of
9 *Megasphaera elsdenii* in the fermentation of
10 DL-[2-¹³C] lactate in the rumen of dairy
11 cattle. Appl. Environ. Microbiol. 42: 649-655.
12
13 8. Csordas, A. (1996). Butyrate, aspirin, and
14 colorectal cancer. Europ. J. Cancer Prevent.,
15 5, 221-231.
16
17 9. Day, A.S. and Abbott, G.D. (1999). D-lactic
18 acidosis in short bowel syndrome. New Zealand
19 Med. J. 112: 277-278.
20
21 10. Duncan, S.H., Hold, G.L., Barcenilla, A.,
22 Stewart, C.S. and Flint, H.J. (2002).
23 *Roseburia intestinalis* sp. nov., a novel
24 saccharolytic, butyrate producing bacterium
25 from human faeces. Int. J. System. Evol.
26 Microbiol., 52, 1-6.
27
28 11. Gilmour, M., Flint, H.J. and Mitchell, W.J.
29 (1994). Multiple lactate-dehydrogenase
30 activities of the rumen bacterium *Selenomonas*
31 *ruminantium*. Microbiol., 1440, 2077-2084.
32

- 1 12. Hold, G.L., Pryde, S.E., Russell, V.J.,
2 Furrie, E. and Flint, H.J. (2002). The
3 assessment of microbial diversity in human
4 colonic samples by 16S rDNA sequence analysis.
5 FEMS Microbiol. Ecol., 39, 33-39.
6
- 7 13. Hove, H., Nordgraard-Andersen, I. and
8 Mortensen, B. (1994). Faecal DL-lactate
9 concentration in 100 gastrointestinal
10 patients. Scand. J. Gastroenterol., 29, 255-
11 259.
12
- 13 14. Hove, H., Holtug, K., Jeppesen, P.B. and
14 Mortensen, P.B. (1995). Butyrate absorption
15 and lactate secretion in ulcerative colitis.
16 Dis. Colon Rect., 38, 519-525.
17
- 18 15. Inan, M.S., Rasoulpour, R.J., Yin, L.,
19 Hubbard, A.K., Rosenberg, D.W. and Giardina,
20 C. (2000). The luminal short-chain fatty acid
21 butyrate modulates NF kappa B activity in a
22 human colonic epithelial cell line.
23 Gastroenterol. 118: 724-734.
24
- 25 16. Kanauchi, O., Fujiyama, Y., Mitsuyama, K.,
26 Araki, Y., Ishii, T., Nakamura, T., Hitomi,
27 Y., Agata, K., Saiki, T., Andoh, A., Toyonaga,
28 A., and Bamba, T. (1999). Increased growth of
29 *Bifidobacterium* and *Eubacterium* by germinated
30 barley foodstuff, accompanied by enhanced
31 butyrate production in healthy volunteers.
32 Int. J. Mol. Med., 3, 175-179.

- 1 17. Kung, L.M. and Hession, A.O. (1995).
2 Preventing in vitro lactate accumulation in
3 ruminal fermentations by inoculation
4 *Megasphaera elsdenii*. J. Anim. Sci., 73, 250-
5 256.
6
7 18. Nocek, J.E. (1997). Bovine acidosis:
8 implications on laminitis. J. Dairy Sci., 80,
9 1005-1028.
10
11 19. Ouwerkerk, D., Klieve, A.V. and Forster, R.J.
12 (2002). Enumeration of *Megasphaera elsdenii* in
13 rumen contents by real-time Taq nuclease
14 assay. J. Appl. Microbiol., 92, 753-758.
15
16 20. Pryde, S.E., Duncan, S.H., Hold, G.L.,
17 Stewart, C.S. and Flint, H.J. (2002). The
18 microbiology of butyrate formation in the
19 human colon. FEMS Microbiol. Letts., 217, 133-
20 139.
21
22 21. Russell, J.B. and Rychlik, J.L. (2001).
23 Factors that alter rumen microbial ecology.
24 Science, 292, 1119-1122.
25
26 22. Suau, A., Bonnet, R., Sutren, M., Godon, J.J.,
27 Gibson, G.R., Collins, M.D. and Dore, J.
28 (1999). Direct analysis of genes encoding 16S
29 rRNA from complex communities reveals many
30 novel molecular species within the human gut.
31 Appl. Environ. Microbiol., 65, 4799-4807.
32

- 1 23. Schwiertz, A., Hold, G.L., Duncan, S.H.,
2 Gruhl, B., Collin, M.D., Lawson, P.A., Flint,
3 H.J. and Blaut, M. (2002). *Anaerostipes caccae*
4 gen. nov., sp. nov., a new saccharolytic,
5 acetate-utilising, butyrate-producing
6 bacterium from human faeces. Syst. Appl.
7 Microbiol., 25, 46-51.
8
9 24. Ushida, K., Hashizume, K., Tsukahara, T.,
10 Yamada, K. and Koyama, K. (2002). *Megasphaera*
11 *elsdenii* JCM 1772^T regulates hyper lactate
12 production in the rat large intestine. Reprod.
13 Nutr. Develop., 42 (Suppl. 1) S56-S57.
14
15 25. Wiryawan, K.G. and Brooker, J.D. (1995).
16 Probiotic control of lactate accumulation in
17 acutely grain-fed sheep. Austral. J. Agric.
18 Res., 46, 1555-1568.
19
20
21



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